Peripheral membrane protein association with lipid bilayers using the Insplorion Acoulyte for combined NPS and QCM-D measurements

The combination of two label-free, surface sensitive measurement techniques based on different physical principles - Insplorion's NanoPlasmonic Sensing (NPS) and Q-Sense quartz crystal microbalance with dissipation monitoring (QCM-D) - enables a detailed study of the dynamics between proteins and lipid bilayers. Here, this is demonstrated by investigating the association of cytochrome *c* with lipid bilayers of different compositions.

Introduction

Membrane-associated proteins play crucial roles in how cells interact with and react to outside stimuli. For example, they control the movement of substances into and out of cells and organelles and have vital roles in cell adhesion and cell signalling. In some cases, membrane-associated proteins can represent more than 50% of the total membrane mass and up to 20% of the total protein content of the cell.

Studying how lipid bilayers interact with proteins and other small molecules can provide valuable insights for the development of model membranes and in the research of biomembrane interactions, as well as provide interesting tools to develop diagnostics and drug delivery systems.

One important aspect of lipid bilayer-protein interaction is the charge of the proteins and the lipids making up the bilayer. Different lipidic compositions produce different total membrane charge, which are expected to
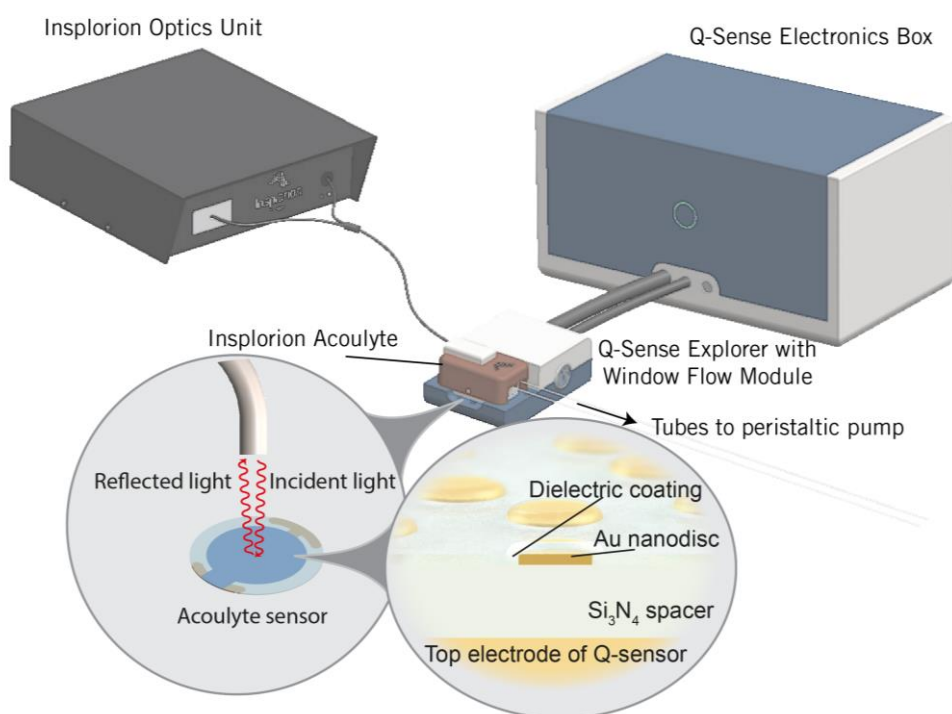

Figure 1. The Insplorion Acoulyte module mounted on a Q-Sense E1/Explorer instrument.

influence how charged molecules interact with the membrane. Not only directly, by influencing the mechanism of interaction, but also indirectly, by changing how much water and salts are retained at the surface of the membranes.

Here, it is shown how combined NPS and QCM-D measurements can be used to study membrane-protein interactions. The complementarity in the measured quantities ("dry"

(optical) vs. "wet" (acoustic) mass) is used to obtain detailed information on the interaction of lipid bilayers, with different lipidic compositions and charges, with membrane-associated protein cytochrome *c*.

Experimental Procedure

The experiments were performed using the Insplorion Acoulyte system with Si_3N_4-coated sensors, which had been oxidized to produce a thin SiO_2 top-layer.
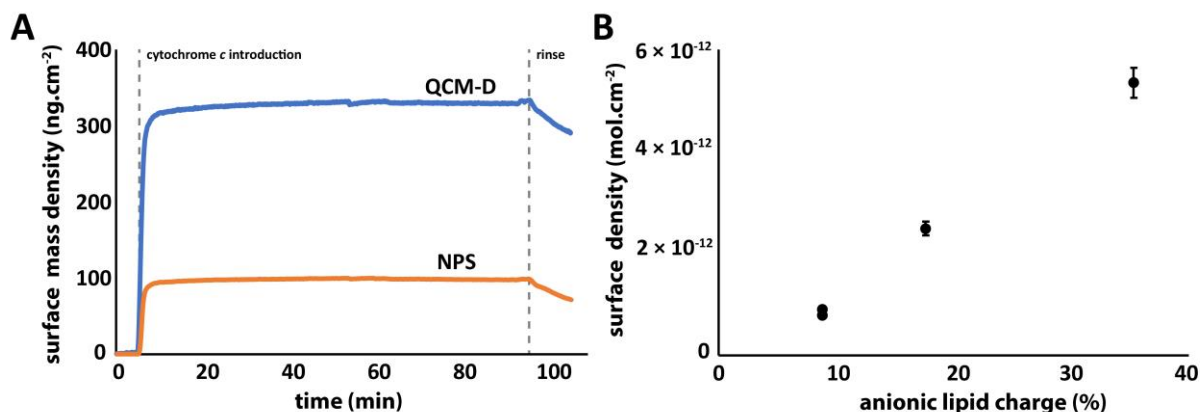
Figure 2. (A) Optical and acoustic mass densities for cytochrome *c* (50 mg/L in 0.01 M NaCl pH 7.4 with 0.01 M HEPES buffer) association with a model lipid bilayer comprised of DOPC and TOCL. The masses were derived from the NPS data (brown line) using the Feijter formula and QCM-D data (orange line) using the Sauerbrey equation, respectively. (B) Surface density of cytochrome *c* molecules as a function of anionic lipid charge in model membranes, with error bars showing the standard deviation of triplicate measurements.

In a first step, lipid bilayers consisting of different ratios of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18:1 phosphatidylcholine, DOPC) and bovine liver α-phosphatidylinositol (Liver PI, a mixture of phosphatidylinositol lipids varying in acyl chain length, degree of saturation, and position of double bonds) or 1',3'-bis[1,2-dioleoyl-sn-glycero-3-phospho]-*sn*-glycerol (18:1 cardiolipin, TOCL) were formed from small unilamellar vesicles, on the sensor surfaces. Horse heart cytochrome *c* (50 mg/L in 0.01 M NaCl pH 7.4 with 0.01 M HEPES buffer) was then flowed over the sensors (0.1 mL/min) while recording the NPS and QCM-D signals.

Results

Figure 2A shows the changes in cytochrome *c* mass density on the surface of the sensors covered with a DOPC lipid bilayer containing 17.6 mol% of TOCL. Despite showing the same kinetic profile, the NPS and QCM-D measurements show a large difference in the surface mass density calculated, indicating a substantial mass of water associated with the cytochrome *c* during the interaction with the lipid bilayer.

The surface mass density of cytochrome *c* on bilayers varying in the amount of TOCL or Liver PI, and thus different anionic lipid charge, is shown in Fig 2B. The cytochrome *c* surface mass density was calculated, from the NPS data, after saturation. There is a direct correlation between the amount of cytochrome *c* associated with the lipid bilayers and the ionic charge of the latter. This is most likely because the presence of a higher negative charge enables more of the highly positively charged cytochrome *c* molecules to associate with the lipid bilayer.

Conclusions

Here, the Insplorion Acoulyte was used to study the interaction of lipid bilayers with a membrane-associated protein. Combined NPS and QCM-D measurements allowed for the quantification of the water content connected to this association. Furthermore, NPS allowed for the assessment of the importance of the ionic charge in the association of cytochrome *c* with lipidic bilayers.

Reference

[1] E.S. Melby et al. *Peripheral Membrane Proteins Facilitate Nanoparticle Binding at Lipid Bilayer Interfaces*.
DOI: 10.1021/acs.langmuir.8b02060

Insplorion AB • Medicinaregatan 8A • 413 90 Gothenburg • Sweden
Tel: +46 (0)31 380 26 95
Web: www.insplorion.com
E-mail: info@insplorion.com